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Helminths of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) from southernmost Brazil

Helmintos of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) do extremo sul do Brasil

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Abstract

Information about helminths of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) are scarce; in this sense the objective of this paper was to contribute to its knowledge. Five hosts of southern Brazil were examined and the helminths *Prosthogonimus ovatus, Tanaisia valida* (Digenea), *Diplotriaena bargusinica* and *Synhimantus* (*Dispharynx*) *nasuta* (Nematoda) were identified. The species *T. valida*, *P. ovatus* and *S.* (*D.*) *nasuta* are for the first time registered for the bird in Brazil. *Prosthogonimus ovatus, T. valida*, *D. bargusinica* e *S.* (*D.*) *nasuta* are first recorded in *M. bonariensis* in the southern Brazilian state Rio Grande do Sul.

Keywords: Shiny cowbird, Trematoda, Nematoda, parasites.

Resumo

Informações sobre helmintos de *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) são escassas, nesse sentido, o objetivo do trabalho foi contribuir para esse conhecimento. Foram examinados cinco hospedeiros do extremo sul do Brasil, identificou-se os helmintos *Prosthogonimus ovatus, Tanaisia valida* (Digenea), *Diplotriaena bargusinica* e *Synhimantus* (*Dispharynx*) *nasuta* (Nematoda). As espécies *T. valida*, *P. ovatus* e *S.* (*D.*) *nasuta* são pela primeira vez registradas para a ave no Brasil. *Prosthogonimus ovatus, T. valida*, *D. bargusinica* e *S.* (*D.*) *nasuta* são pela primeira vez registradas em *M. bonariensis* no estado do Rio Grande do Sul.

Palavras-chave: Vira-bosta, Trematoda, Nematoda, parasitos.

Parasites are among the most common organisms in the world (HOBERG & KUTZ, 2013). They are extremely diversified and play a key role in ecological and evolutionary processes (GOMÉZ & NICHOLS, 2013). Parasitism is one of the most successful ways of living presented by living organisms (POULIN & MORAND, 2000).

Birds are commonly parasitized by trematodes, nematodes, cestodes and acanthocephalans, as well as other groups (AMATO & AMATO, 2010). Taking into account the fact that Brazil presents one of the richest avifaunas in the world, much remains to be explored regarding this subject.

Molothrus bonariensis (Gmelin, 1789) (Passeriformes: Icteridae), known as the shiny cowbird, occurs in South America, islands of Central America, United States and Canada (The IUCN Red List of Threatened Species, 2015). In the state of Rio Grande do Sul, Brazil, they inhabit the entire state, where enormous flocks often gather in cereal crops, especially rice, during and after harvesting (BELTON, 1994). The male is black-feathered with bluish reflexes, and the female is brown. They feed on seeds and insects, and occasionally on fruits, and often can be seen near to cattle, turning over excrement to look for undigested seeds (EFE et al., 2001).

Information regarding the shiny cowbird is mainly focused on the fact that this species lays its eggs in other birds' nests. In Brazil, approximately 60 species belonging to nine families of Passeriformes are affected by the action of the shiny cowbird. This species is entirely dependent on other bird species for its reproduction, because it has not only lost the ability to build its own nests but also the instinct of taking care of its offspring (BLANCO, 1995; SICK, 1997; EFE et al., 2001; MERMOZ & REBOREDA, 2003; ASTIÉ & REBOREDA, 2006).

Regarding helminths in *M. bonariensis* in Brazil, there have been isolated reports of *Lyperosomum oswaldoi* (Travassos, 1919) (Trematoda: Dicrocoeliidae) (TRAVASSOS et al., 1969), *Diplotriaena bargusinica* Skrjabin, 1917 (Nematoda: Diplotriaenidae) (VICENTE et al., 1983) and *Mediorhynchus emberizae* (Rudolphi, 1819) (Acanthocephala: Gigantorhynchidae) (PETROCHENKO, 1971). In this context, the present study had the objective of reporting the presence of helminths associated with *M. bonariensis.*

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Five hosts were examined (one male and four females), originating from the municipality of Rio Grande, Rio Grande do Sul, Brazil (32º 14'.37.24" S; 52°29'38.71" W) two birds in the summer and three in the winter. They were accidentally sampled in a trap that had been set to catch *Chrysomus ruficapillus* (Vieillot, 1819) (Passeriformes: Icteridae), in accordance with a license granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio No. 41095-3) and approval granted by the Comitê de Ética e Experimentação Animal from the Universidade Federal de Pelotas (CEEA/UFPel No. 147), covering the period of 2013-2015.

These specimes of *Molothrus bonariensis* were euthanasied, individually placed in identified plastic bags, and were transported to the Laboratório de Parasitologia de Animais Silvestres (LAPASIL/UFPel) and frozen until processing. To collect helminths, were necropsied and their organs (mouth, esophagus, proventriculus, gizzard, cecum, small and large intestines, trachea, lungs, heart, liver, gall bladder, pancreas, reproductive system, kidneys, cloaca and air sacs) were separated from each other, opened and rinsed with running water under a sieve of mesh size 150 µm.

The helminths were fixed in AFA for 24 hours and were stored in accordance with the protocol proposed by Amato & Amato, 2010. Trematodes were stained with Langeron's carmine and nematodes were clarified with Aman's lactophenol. They were mounted on permanent and semi-permanent slides and photographed through a microscope (Olympus® BX 41) with a coupled camera system. Morphological and morphometric identification were performed in accordance with Freitas (1951), Kohn & Fernandes (1972), Gibson et al. (2002), Bray et al. (2008), Lunaschi et al. (2015) for Digenea, and with Vicente et al. (1983), Zhang et al. (2004) and Anderson et al. (2009), for Nematoda. The parameters calculated were prevalence (P), mean abundance (MA) and mean intensity of infection (MI), in accordance with Bush et al. (1997). The voucher specimens were deposited in the "Coleção de Helmintos do Laboratório de Parasitologia de Animais Silvestres" from Instituto de Biologia, Universidade Federal de Pelotas (CHLAPASIL/UFPel) (No. 612 to 616).

Out of the five hosts *Molothrus bonariensis* examined four were positive for helminths (only females). Were identified two species of Trematoda *Prosthogonimus ovatus* (Rudolphi, 1803) (Lühe, 1899) (Prostogonimidae) (n = 5) and *Tanaisia valida* Freitas, 1951 (Eucotylidae) (n = 20) and two Nematoda *Diplotriaena bargusinica* Skrjabin, 1917 (Diplotriaenidae) (n = 37) (20 males and 17 females) and *Synhimantus* (*Dispharynx*) *nasuta* (Rudolphi, 1819) Chabaud, 1975 (Acuariidae) (n = 2) (one male and one female), the results for each host and the parasitological indexes are presented in Table 1. *Diplotriaena bargusinica* was the most prevalent parasite and the one with the highest mean infection intensity, followed by *T. valida*. The trematode *P. ovatus* and *T. valida* co-occurred (one host); *D. bargusinica* and *T. valida* (one host) and *D. bargusinica* and *S.* (*D.*) *nasuta* (one host). There were not analyzed pathological aspects related to the identified helminths.

The morphological identification of each species was carried out according to the characteristics described by the previously mentioned authors. We present below the relevant aspects of each helminth. *P. ovatus* (Figure 1) is characterized by having the piriformis body, subterminal oral sucker, muscular pharynx present (Figure 2), simple intestinal cecum, few meandering surpassing the testicles. Acetabulum pre-equatorial, terminal genital pore located next to the oral sucker. Cirrus sac extending from the genital pore to the level of esophageal bifurcation, well-developed testicles, post-acetabular, post-ovarian, equatorial (Figure 3). Ovary pretesticular lobed, Mehlis gland situated below the ovary, uterus occupies half of the body and the excretory portion is terminal.

Tanaisia valida has elongated body, flattened, in tegument with thorns, vitelline glands begging in post-ovarian region to the back of the body (Figure 4), oral sucker subterminal, non visible acetabulum, muscular pharynx (Figure 5), intestinal cecum is positioned dorsally in relation to the gonads fused near to end of the body. Lobate testis with diagonal layout, pre-equatorial, post-ovarian (Figure 6). Lobate ovary, uterus with a large number of eggs, these yellow-brownish color with a smooth shell.

In the anterior region of *D. bargusinica* has a trident well developed (Figure 7), trident with tapered apex (Figure 8), females has a genital opening near to the anterior region (Figure 9), eggs with size range of 0.044 × 0.029mm (Figure 10), females with posterior rounded region (Figure 11), males with uneven spicules with small number of papillae (Figure 12).

Synhimantus (*D.*) *nasuta* (Figure 13) has a filiform body, cuticle striated crosswise, small and conic lips, four cords recurrent non anastomosing beginning on surface dorsal and ventral of the oral opening extending to the posterior portion of the muscle esophagus and bifurcated cervical papillae (Figure 14) Females presented well-developed uterine, vulvar opening near the posterior end of the body (Figure 15). Males presented curved caudal end, nine pairs of caudal papillae, four pairs pre-cloacal and five post-cloacal (Figure 16), unequal and dissimilar spicules (Figure 17).

The trematodes Prosthogonimidae (Lühe, 1909) have worldwide distribution. They are parasites of the bursa of Fabricius, oviduct,

Table 1. Helminths and parasitological indexes of *Molothrus bonariensis* (Passeriformes: Icteridae) are from Rio Grande, Rio Grande do Sul, Brazil.

Helminths	Host	Total helminths/Host	P (%)	MA	MI
Trematoda					
Prosthogonimus ovatus (Rudolphi, 1803)	Mb*3	5	20	1	5
Tanaisia valida Freitas, 1951	Mb*3, Mb*4	11; 9	40	4	10
Nematoda					
Diplotriaena bargusinica Skrjabin, 1917	Mb*2, Mb*4, Mb*5	18; 18; 1	60	7.4	12.3
Synhimantus (*D.*) *nasuta* Skrjabin, 1917 Chabaud, 1975	Mb*5	2	20	0.4	2

Mb=*Molothrus bonariensis*; *Female; Mb1=host negative.

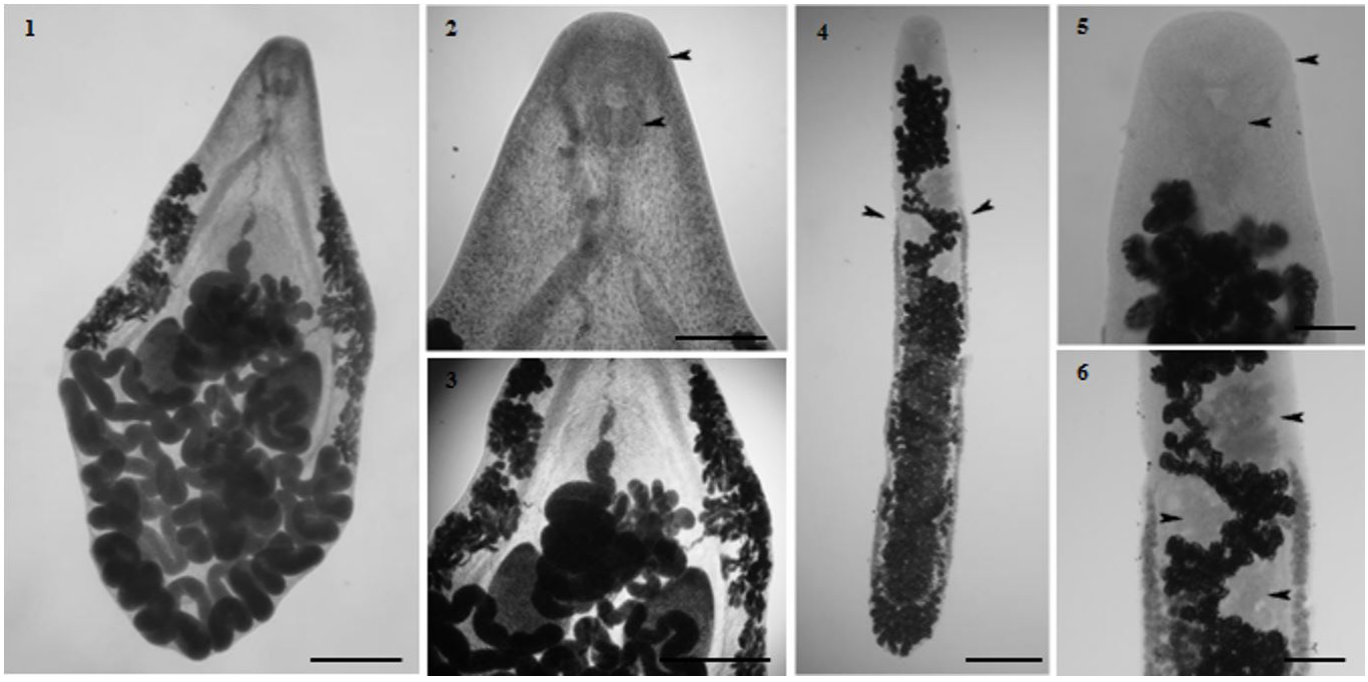

Figure 1-6. 1-3- *Prosthogonimus ovatus* (Rudolphi, 1803) (Lühe, 1899) (Trematoda: Prostogonimidae) of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) from southern Brazil. 1- ventral view of *P. ovatus;* bar = 0.6 mm; 2- arrows point to the oral suckers and pharynx; bar = 0.25 mm; 3- median area of the body, vitellarias, ovary and testicles; bar = 0.68 mm. 4-6 *Tanaisia valida* Freitas, 1951 (Trematoda: Eucotylidae) of *M. bonariensis*. 4- ventral view of *T. valida*; Arrows point out the beginning of the vitelline glands; bar = 0.39 mm; 5- anterior area, arrows point to the terminal oral sucker and pharynx; bar = 0.08 mm; 6- median area of the body, arrows point to ovary and lobate testes; bar = 0.14 mm.

cloaca or nictitating membrane of birds, in mammals are found in the intestine, liver and body cavity (BRAY et al., 2008).

In Brazil, *P. ovatus* were registered in the bursa of Fabricius of *Gallus gallus domesticus* (Linnaeus, 1758) (Galliformes: Phasianidae) (n = 17) P = 17.6% and in wild birds: *Theristicus caerulescens* (Vieillot, 1817) (n = 6) P = 16.6%; *Mesembrinibis cayennensis* (Gmelin, 1789) (n = 3) P = 33.3% (Pelecaniformes: Threskiornithidae); *Piaya cayana* (Linnaeus, 1766) (Cuculiformes: Cuculidae) (n = 3) P = 33.3%; *Monasa nigrifrons* (Spix, 1824) (Galbuliformes: Bucconidae) (n = 10) P = 10.0% *Cyanocorax cyanomelas* (Vieillot, 1818) (Passeriformes: Corvidae) (n = 4) P = 25.0% *Xiphorhynchus guttatus* (Lichtenstein, 1820) (Passeriformes: Dendrocolaptidae) (n = 1) P = 100% (TRAVASSOS, 1928; TRAVASSOS et al., 1969).

Kohn & Fernandes (1972) reported the ocurrence of *P. ovatus* in *Pteroglossus aracari* (Linnaeus, 1758) (Piciformes: Ramphastidae), *Cairina moschata* (Linnaeus, 1758) and *Cairina moschata domesticus* (Anseriformes: Anatidae), *Crotophaga major* Gmelin, 1788 (Cuculiformes: Cuculidae), *Theresticus caerulescens* (Vieillot, 1817) (Pelecaniformes: Threskiornithidae), *Hydropsalis* Wagler, 1832 (Caprimulgiformes: Caprimulgidae), *Leptoptila verreauxi* Bonaparte, 1855 (Columbiformes: Columbidae), in the Charadriiformes: *Thalasseus maximus* (Boddaert, 1783) (Sternidae), *Gallinago paraguaiae* (Vieillot, 1816) (Scolopacidae), *Larus* Linnaeus, 1758 and *Larus dominicanus* (Laridae) in Passeriformes: *Tangara sayaca* (Linnaeus, 1766) and *Lanio melanops* (Vieillot, 1818) (Thraupidae), *Turdus amaurochalinus* Cabanis, 1850 (Turdidae), *Taraba major* Gmelin, 1788 (Thamnophilidae), *Cyanocorax chrysops* (Vieillot, 1818) (Corvidae), *Icterus croconotus* (Wagler, 1829) and *Sturnella superciliaris* (Bonaparte, 1840) (Icteridae).

In Rio Grande do Sul, Monteiro et al. (2007) identified *P. ovatus* in waterbirds, *Dendrocygna bicolor* (Vieillot, 1816) (n = 33), P = 3.0% and MI = 1; and *Netta peposaca* (Vieillot, 1816) (n = 20), P = 15.0% and MI = 4.3 (Anseriformes: Anatidae); and in *Phalacrocorax brasilianus* (Gmelin, 1789) (n = 47), P = 2.1% and MI = 1 (Suliformes: Phalacrocoracidae). Mascarenhas et al. (2009) identified it in *Paroaria coronata* (Miller, 1776) (n = 40), P = 7.5% and MI = 3 (Passeriformes: Thraupidae).

According to Boddeke (1960) the life cycle of *P. ovatus* involves two intermediate hosts, the first is a mollusk and the second are young forms of Odonata. The bird infection occurs through ingestion of young or adult dragonflies containing the encysted metacercariae (BODDEKE, 1960). In laying birds, they affect egg production, through causing a decline in egg formation, or non-formation (OLSEN, 1974).

Tanaisia Skrjabin, 1924, parasitizes the renal tubules and kidneys of birds. In Brazil, it has mainly been reported in Passeriformes, although there have been records of occurrences in Cuculiformes, Charadriiformes, Gruiformes and Strigiformes. The Eucotylidae taxonomy is a complex one due to morphological structures that characterize the genera and species (integument, position and shape of the testis and ovaries, etc.).

Lunaschi et al. (2015) redescribed *Tanaisia dubia* Freitas, in 1951, had created a key to the Neotropical species *Tanaisia* and a key to the Tanaisiinae genres that gives support in species

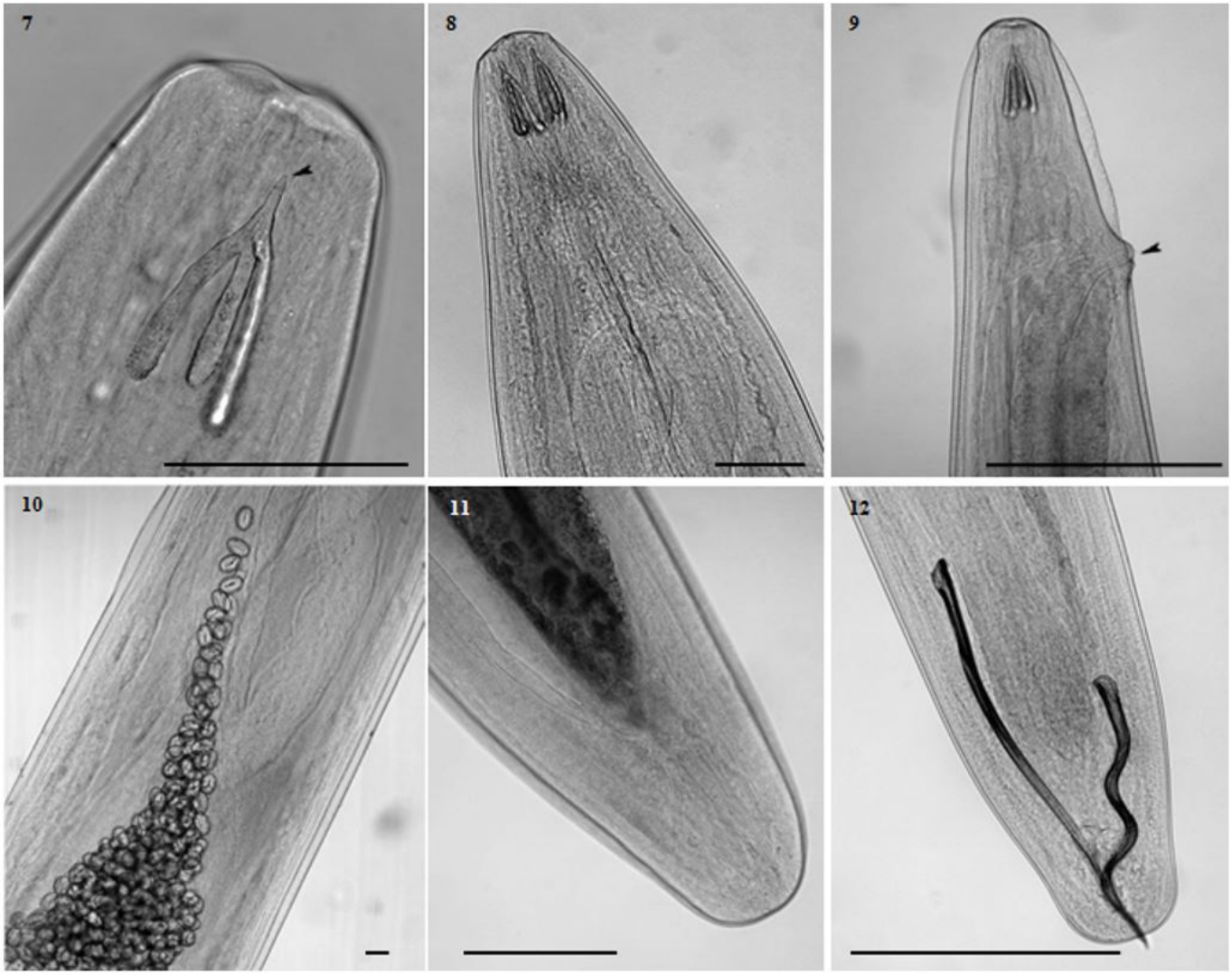

Figure 7-12. *Diplotriaena bargusinica* Skrjabin, 1917 (Nematoda: Diplotriaenidae) of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) from southern Brazil. 7- dorsal view, smooth trident highlighted, arrow points to the apex of the tapered trident. bar = 0.14 mm; 8- dorsal view of the anterior area; bar = 0.14 mm; 9- anterior area of the female, arrow points to genital opening; bar = 0.35 mm; 10- posterior area of the male, detail of bigger and smaller spicules; bar = 0.5 mm; 11- posterior area of the female; bar = 0.25 mm; 12- median area of the female, detail of the eggs; bar = 0.05 mm.

identification. Those authors pointed out the extent of vitelline glands as an important morphological characteristic to distinguish three genres of Tanaisiinae, and in the species of *Tanaisia* the vitelline glands extend up from ovarian or testicular region.

Tanaisia valida Freitas, 1951 was described in *Himantopus melanurus* Vieillot, 1817 (Charadriiformes: Recurvirostridae) in the state of Rio de Janeiro and, later on, was reported by Mascarenhas et al. (2009), in *Paroaria coronata* (Miller, 1776) (Passeriformes: Thraupidae) (n = 40), with P = 2.5%, MA = 0.05 and MI = 2, in the state of Rio Grande do Sul.

In South America, other authors have had identified *T. valida* in Charadriiformes. In Peru, Ibañez-Herrera (1998) recorded *T. valida* parasitizing kidneys in *Himantopus melanurus* Vieillot, 1817 (Recurvirostridae) without presenting parasitological and sample size indexes, because it is a list of species for the fauna of Peru, later Tantalean et al. (1992) recorded in *Phalaropus tricolor* (Vieillot, 1819) (Scolopacidae) in the same country. *Tanaisia valida* was identified in *Charadrius wilsonia* Ord, 1814 (Charadriidae) (n = 1) (P = 100%) (MI = 4) in Colombia (LUNASCHI et al. 2015).

About the life cycle of *T. valida* does not exist information, however considering the life cycle of Tanaisiinae, the birds become infected by ingesting (Mollusca: Gastropoda) containing metacercariae (LUNASCHI et al. 2015).

Diplotriaena Railliet & Henry, 1909, has parasitic specificity restricted to birds and is distributed among Anseriformes, Apodiformes, Galliformes, Charadriiformes, Columbiformes, Piciformes and Passeriformes, with wide geographic distribution (VICENTE et al., 1983; ATKINSON et al., 2009).

In Brazil *D. bargusinica* was reported by Vicente et al. (1983) parasitizing some Passeriformes in different areas of the country: *Cacicus cela* (Linnaeus, 1758), *Cacicus haemorrhous* (Linnaeus, 1766),

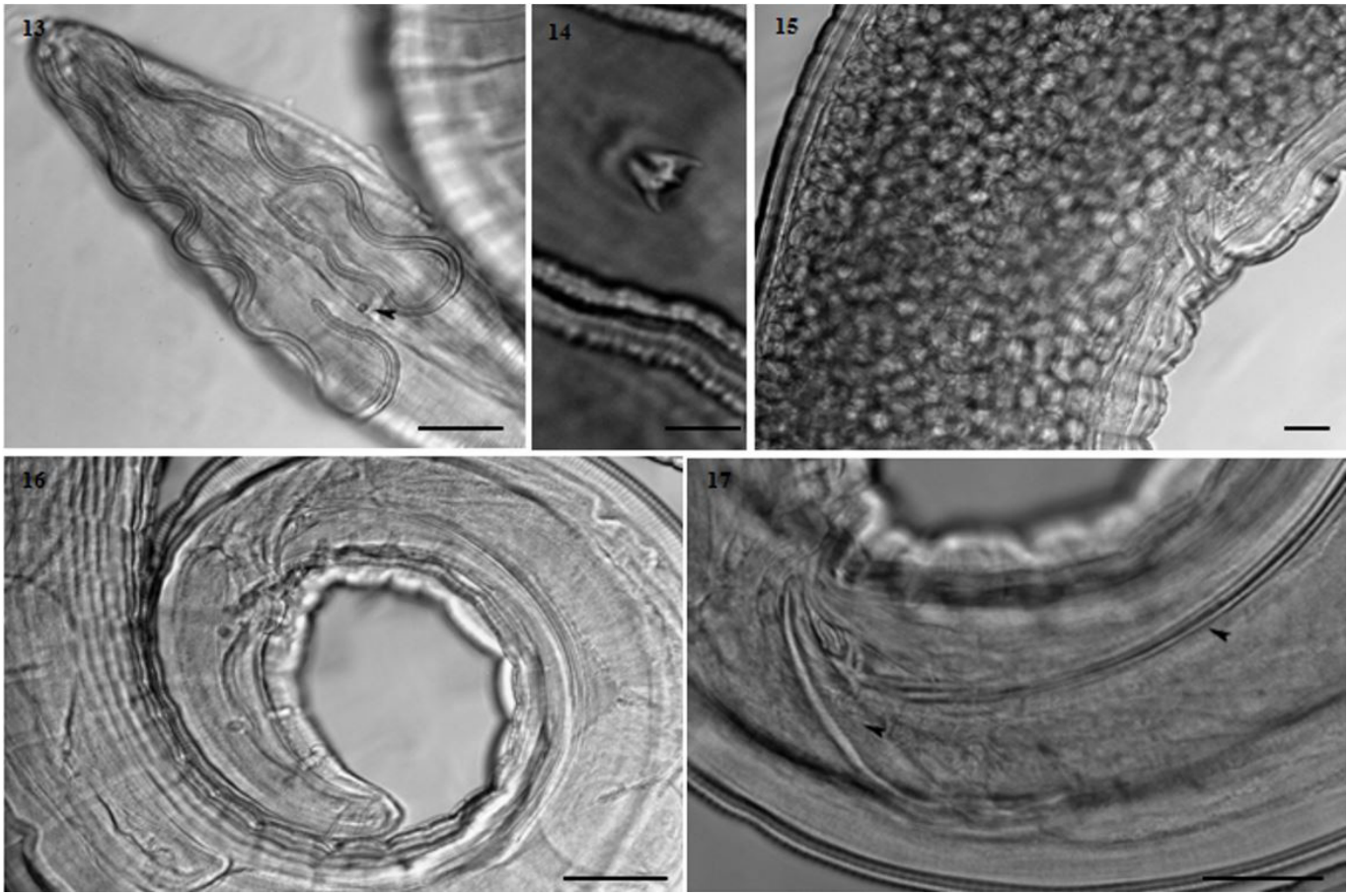

Figure 13-17. *Synhimantus (Dispharynx) nasuta* (Rudolphi, 1819) Chabaud, 1975 (Nematoda: Acuariidae) of *Molothrus bonariensis* (Gmelin, 1789) (Passeriformes: Icteridae) from southern Brazil. 13- recurrent cordons of *S.* (*D.*) *nasuta*, arrow points to the papilla; bar = 0.07 mm; 14- detain of the bifurcated cervical papilla; bar = 0.01 mm; 15- genital opening of the female; bar = 0.03 mm; 16- posterior area of the male, detail of pre and post-cloacal papillae; bar = 0.17 mm; 17- lateral view, arrows point to uneven spicules; BAR = 0.8 mm.

Gnorimopsar chopi (Vieillot, 1819), *Icterus croconotus* (Wagler, 1829), *Icterus* sp., *Psarocolius decumanus maculosus* (Chapman, 1920), *Molothrus bonariensis* (Gmelin, 1789) (Icteridae) and *Xiphocolaptes major castaneus* Ridgway, 1890 (Dendrocolaptidae), in the states of Mato Grosso do Sul, São Paulo and Pará and in Paraguay, without informing the number of birds examined or their indexes.

Pinto et al. (1997) reported the presence of *D. bargusinica* for the first time in *Cyanocorax cyanomelas* (Vieillot, 1818) (Corvidae) in the state of Mato Grosso do Sul, from samples collected between 1921 and 1925 in scientific expeditions conducted in Brazil, without informing the number of birds examined or their indexes. Gonçalves et al. (2002) found *D. bargusinica* in the body cavity of three individuals of *Psarocolius bifasciatus* (Spix, 1824) (Icteridae) in the state of Amazonas and Carvalho et al. (2007) in air sacs of *Paroaria dominicana* (Linnaeus, 1758) (Emberizidae) (n = 34), P = 8.8%, MA = 0.32 and MI = 3.66, in the state of Bahia.

Anderson (1962), performed experimental infection with *D. bargusinica* in wild birds (Turdidae and Icteridae) and described details of its biological cycle which uses grasshoppers (Orthoptera) as intermediate hosts. Birds become infected by ingesting grasshoppers that contain third-stage larvae, and the adult nematodes become established in the air sacs of the host (ANDERSON, 2000). The clinical signs common to parasitized birds are lethargy, labored breathing, size and weight below average and little plumage (ATKINSON et al., 2009).

Synhimantus (*D.*) *nasuta* has worldwide distribution and parasitizes the proventriculus and gizzard of birds. In Brazil, it has been found in Galliformes of economic importance, and also in Columbiformes, Cuculiformes, Strigiformes, Falconiformes, Charadriiformes and Passeriformes (BARTMANN & AMATO, 2009).

In Rio Grande do Sul, *S.* (*D.*) *nasuta* was reported in *Paroaria coronata* (Miller, 1776) (Passeriformes: Thraupidae) (n = 40), P = 2.5%, MA = 0.1 and MI = 4, by Mascarenhas et al., 2009; in *Guira guira* (Gmelin, 1788) (n = 120), P = 28.3%, MA = 2.28 and MI = 8.8; in *Crotophaga ani* Linnaeus, 1758 (Cuculiformes: Cuculidae) (n = 120), P = 26.7%, MA = 1.32 and MI = 5.1, by Bartmann & Amato (2009); in *Columbina picui* (Columbiformes: Columbidae) (n = 34), P = 5.9%, MA = 1.1 and MI = 19.5, by Coimbra et al. (2009); in *Vanellus chilensis* (Molina, 1782) (Charadriiformes: Charadriidae) (n = 28), P = 28.6%, MA = 4.11 and MI = 14.38, by Avancini (2009); in *Passer domesticus* (Linnaeus, 1758) (Passeriformes: Passeridae) (n =

160), P = 0.63%, MA = 0.11 and MI = 18, by Calegaro-Marques & Amato (2010); and in *Pitangus sulphuratus* (Passeriformes: Tyrannidae) (n = 78), P = 3.85%, MA = 0.10 and MI = 2.66, by Mendes (2011).

The biological cycle of *S.* (*D.*) *nasuta* is heteroxenous, using terrestrial isopods, orthopterans, beetles or millipedes (GOBLE & KUTZ, 1945). The larvae develop in non-specific tissues of isopods, in which they become encapsulated and are later on ingested by birds (ANDERSON, 2000). The effects on the definitive host are related to the parasitic load, usually comprising inflammations, granulomas and lesions in the proventriculus. In wild birds, these macroscopic and histopathological alterations were described by Bartmann & Amato (2009).

In general, the parasitological indexes of *M. bonariensis* are similar to those found by those previously mentioned authors, considering that the previous studies had sample sizes close to the one of the present study future research is needed to complement existing information in order to contribute to the understanding of the biology and ecology of these organisms.

In Brazil, for the first time the species *T. valida*, *P. ovatus* e *S.* (*D.*) *nasuta* are recorded parasitizing *M. bonariensis*. *Prosthogonimus ovatus*, *T. valida*, *D. bargusinica* e *S.* (*D.*) *nasuta* characterize first record in *M. bonariensis* in Rio Grande do Sul. Whereas the helminthological information for the specie are scarce, this work expands the knowledge to Icteridae and *Molothrus bonariensis*.

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